I. Report of Genetics Subcommittee

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Progress towards 2008 Global Priorities

The following global priorities and resources were identified by the 2008 IBD Diagnoses working group:

- Identify additional CD-associated genetic variants through joint analysis and deeper replication studies of existing genome-wide association data
- Perform genome-wide association studies in UC, early-onset, and minority racial/ethnic IBD cohorts
- Determine the functional mechanisms of IBD genes
- Develop and apply statistical and experimental approaches to identifying gene–gene and gene–environment interactions
- Determine the predictive value of IBD-associated genetic variants for development of IBD, disease subtype and course, and response to therapies

Since 2008 significant and rapid progress has been made with regard to the identifying additional Loci in CD and UC in European extracted populations. Arguably, no other field in IBD has advanced as quickly as the identification of susceptibility loci. CD and UC have seen notable successes culminating in the discovery of over 100 published susceptibility loci/genes to date. The majority of loci described confer susceptibility to both CD and UC including multiple genes involved in IL23/Th17 signaling (IL23R, IL12B, JAK2, TYK2 and STAT3), as well as IL10, IL1R2, REL, CARD9, NKX2.3, ICOSLG, PRDM1, SMAD3 and
ORMDL3. The evolving genetic architecture of IBD has furthered our understanding of disease pathogenesis. For CD, defective processing of intracellular bacteria has become a central theme, following gene discoveries involved in autophagy and innate immunity (e.g. NOD2, IRGM, ATG16L1). Genetic evidence has also demonstrated the importance of barrier function to the development of UC (HNF4A, LAMB1, CDH1 and GNA12). Another emerging theme is the overlap of susceptibility loci with other immune related diseases paralleling the reported associations from epidemiological studies. However, minimal progress has been made to date with identifying novel and additional susceptibilities in very early onset IBD (onset less than 8 years old), and non-European derived IBD cohorts.

**Top Research Priorities**

The group felt that the most important research questions in the area of IBD genetics mainly fall under the banner of translating the discoveries for clinical utility in the management of IBD; including diagnostics, predicting risk, individualizing therapy and therapeutics. Identifying and recruiting multiple affected families for genetic studies and understanding functional mechanisms of gene and their products are also additional top priorities. The most pressing priorities include:

1. **Personalized medicine:** Discovering therapeutic targets, performing expression and SNP analysis to identify genetic and genomic variations associated with natural history and inter-individual differences in drug response. The development and validation of clinically useful models that incorporate genomics and other ‘–omics’ together with clinical observations that can effectively ‘predict’ natural history and discriminate responders and non-responders to pharmacologic and biological interventions in IBD.

The heterogeneous nature of IBD, together with an increased understanding of the genetic architecture of these conditions, lends to a robust personalized approach to clinical management. These approaches
will be greatly facilitated by ever-decreasing genotyping and sequencing costs. A major challenge for geneticists and basic scientists is bridging the ‘benchside to bedside’ divide and the translation of these major advances into the clinical sphere. This translation can occur in a number of different ways including; novel therapeutic approaches (including drug discovery); diagnostics; and prognostics (including pharmacogenomics). The application of pharmacogenomic approaches to therapies used in arthritis and rheumatic diseases holds great promise for "personalized medicine," in which drugs and drug combinations can be tailored to each individual’s unique genetic makeup. This will require the collection and characterization of large, prospective, robustly phenotyped cohort. In addition, if genetic variation is to be used with any confidence for diagnostic and genetic counseling purposes then studying large numbers and extended families drawn from representative populations will be necessary. Prospective longitudinal studies will be needed to determine the predictive value of genetic variants for disease subtype and course and response to therapies. It has been suggested that there are four main barriers to bridging the bench to bedside divide: ‘making genomics-based diagnostics routine’; ‘defining the genetic components of disease’; ‘practical systems for clinical genomic informatics’; and understanding ‘the role of the microbiome in health and disease’. These are areas where researchers should focus their efforts in IBD.

2. Explore the gene and gene product discoveries into biological mechanisms of disease

Genome-wide association (GWA) studies have, through unbiased analyses, transformed the discovery of gene regions, or loci, related to disease risk. Association and linkage association studies have yielded important insights and highlighted relevant pathways in the pathogenesis of CD and UC. These efforts have also revealed common genetic factors contributing to other immune-related and infectious diseases. However, GWA approaches only identify regions that harbor risk genes, requiring follow-up
studies to discover the precise, disease-causing gene variants, or single nucleotide polymorphisms (SNPs). In addition, the SNPs found through genome-wide association studies describe only a small fraction (about 25%) of inherited disease risk. This is likely to be an underestimate, but both of the aforementioned factors require more in-depth analyses of identified susceptibility loci. These approaches will include the use of alternative genomic approaches such as next generation sequencing technologies and exome chip studies, in order to capture and characterize additional rare genetic variants. Once causative variation has been identified or suspected, then a number of techniques can be utilized to understand the implicated biological mechanisms. These techniques include 1) integrating gene expression profiling with genotypic variation, 2) exploring network-based or similar approaches to elucidate the complexity of disease traits, 3) translating complex genetic analyses in animal models to humans, 4) making use of positionally cloned quantitative trait loci, and 5) characterizing the functional effects of genetic variants using robust in vitro and in vivo systems including studies of single genes, multiple genes, gene-gene interactions, and gene-environment interactions. Studies of epigenomics, gene-environment interactions, chromatin structure, copy number variants, and microRNAs have largely been underexplored in the GWAS era and will warrant considerable effort over the coming years.

3. Recruiting well-characterized, multiply affected family-based cohorts (both affected and unaffected individuals) in order to explain both heritable traits and identify rare but high effect variants using combined linkage and association analyses in addition to state of the art sequencing technologies.

Studies to detect genetic association with disease can either be family-based, often using families with multiple affected members, or population based case-control studies. Recently published GWA studies were based on case-control design. Families with a high penetrance of disease are likely to have genetic variants with significant effect sizes (often greater than that seen with more common variation typically
identified by case-control studies). These rarer variants (which may even be ‘private mutations’ to any given family) may be of significant benefit to researchers. These variants may provide insight into the functional consequences of genetic variation in IBD ‘genes’ that may be more difficult to elucidate in more common variation with smaller effect size. It is important to collect data on unaffected family members of IBD subjects as well (such as in the Crohn’s and Colitis Foundation of America (CCFC) Genetics, Environmental, Microbial (GEM) study). This allows for study of heritable traits and serves as a resource of genetically enriched (for IBD genes) individuals available for functional studies where function is unlikely to be affected by drugs and/or disease activity.

4. Study the gene-microbial interactions.

Recent studies have highlighted the role of host genomic variation and its role in determining microbial (both bacterial and viral) patterns. Interest in the role the microbiome in IBD has significantly increased as technological and analytic advances have evolved. The importance of characterizing the microbiome (bacteriome, virome, and fungiome etc) in IBD patients, in both stool and mucosal samples, is covered in more detail in the microbiome section of this document. A highly relevant interface is the effect of human genetic variation on the microbial composition and the effect of the combination of these variables in susceptibility to, and the natural history of, IBD. This is currently being pursued through the CCFA Microbiome Initiative.

Approaches and Resources Required to Address these Priorities

- Support is needed for investigators to leverage and sustain the ongoing cohort studies such as RISK, CCFA PARTNERS (an internet-based IBD cohort of patient reported outcomes) and GEM and their rich data and bio-materials.
Support is needed for both large-scale projects such as the CCFA Genetic Initiative as well as more focused projects on individual genes or pathways often proposed through the Career Development and Senior Awards mechanisms.

Support is urgently needed for a centralized and distributable infrastructure for biobanking, large servers to deposit genomic information, data warehouses, and tissue/cell repositories for integrated human investigation. Ideally this infrastructure will allow access to data and biospecimens collected prior to and following treatment with established and novel therapeutics and to recruit and follow patients in a longitudinal manner.

References:


II. Report of Epidemiology and Environmental Factors Subcommittee

Edward V. Loftus, Jr, MD (Chair), Michael D. Kappelman MD, MPH (Co-Chair), Ashwin N. Ananthakrishnan MD, MPH, Eric I. Benchimol MD, PhD, Ajay S. Gulati MD, Susan Hutfless PhD, Gilaad G. Kaplan MD, MPH, and Millie D. Long MD, MPH.

Summary

- Despite abundant indirect evidence that suggests a role for environmental factors in the pathogenesis IBD, there is limited direct evidence for the role of specific environmental factors in either triggering or protecting against disease onset or progression.

- The field of IBD epidemiology has been limited by methodological challenges, including inconsistent measurement (misclassification) of exposures and outcomes, the inherent difficulty in recruiting and following sufficient numbers of subjects for long enough time periods, and the prior inability to measure and account for gene-environment interactions.

- Emerging pharmacoepidemiological studies have demonstrated the long-term effectiveness of biologic anti-tumor necrosis factor-alpha (anti-TNF) therapy in CD, the relative and absolute risks of unintended outcomes including infection and malignancy (non-Hodgkin’s lymphoma, non-melanoma skin cancer, and melanoma) associated with these and other medications, and preliminary evidence of the safety and effectiveness of these agents in populations not initially studied in randomized trials (e.g., children and pregnant women).
• Pharmacoepidemiological studies of IBD have been limited by the lack of clinical data available in
administrative/health insurance databases, resulting in unmeasured confounding (degree of
perianal disease, depth of ulceration, etc.) and possible misclassification of exposures or outcomes
of interest. Clinical registries and electronic medical record (EMR)-based studies may have greater
clinical details, but often lack capture of events/care occurring outside of tertiary centers or
gastroenterology practices.

Top Research Priorities

1. Epidemiological studies of disease etiology which focus on gene-environment interactions and
incorporate the simultaneous measurement of environmental and genetic factors prior to
disease onset in order to evaluate whether the effects of various exposures vary by host
genetics.

Such studies should also incorporate the collection of relevant biospecimens (e.g., stool for
microbial analysis, barrier function tests, etc.) before and after exposure(s) of interest in order
to facilitate translational studies of disease mechanisms. Given the problems inherent with
sample size and multiple testing, such studies should focus on biologically plausible sets of
candidate genes and related exposures and their downstream effects on host biology, defined a
priori based on currently available knowledge regarding the mechanisms/pathways of known
risk alleles. Emphasis should be placed on modifiable risk factors (either protective or
causative), in order to maximize the impact on population health and prevention. Studies of
high-risk populations might improve efficiency and power, albeit at the risk of generalizability.
Study outcomes must be specific IBD sub-phenotypes rather than generic IBD, as environmental risk factors for these disparate phenotypes may differ.

2. **Epidemiological studies of the natural history of patients diagnosed with IBD to evaluate the role of environmental factors on flares/disease progression.**

Such studies may be either population based or multi-center studies, provided that internal validity can be assured. It is recommended that studies combine collection of environmental exposures with the collection of genetic, clinical, and serological, and other biomarkers to improve control of confounding and enhance precision. Efforts should focus upon identification of the most biologically relevant exposures that might impact natural history/flares/progression of established disease based on current knowledge of gene pathways (diet—specific components, NSAID, vitamin A/D, etc.). Measurement of such factors should be standardized, including formal evaluation of existing instruments and creation and validation of new instruments when needed.

3. **Pharmacoepidemiological studies of the risks and benefits of available treatment options used under real-world conditions and in diverse populations are needed to further inform treatment algorithms.**

Such studies must focus on absolute, rather than relative, risks and benefits and should evaluate treatment strategies (i.e., sequential versus combination therapy)
Approaches and Resources Required to Address these Priorities

- As sample size and length of time required (and expense) to initiate new cohorts to study disease etiology may be prohibitive, initial efforts and resources should concentrate on identifying, evaluating, cataloging, and forming relationships with ongoing North American and European cohorts intended to study other conditions (cancer, obesity, different chronic conditions, etc.). If such cohorts include prospective collection of similar exposure data at similar time points along with genetic data (or blood samples), they might be combined to facilitate studies of gene-environment interaction. Ongoing studies may be enhanced by collection of additional biospecimens (such as stool) to evaluate effects of environmental exposures and host genetics on the microbiota, immune function, barrier function, etc in individuals who do and do not ultimately develop IBD. Initial funds should concentrate on identifying such cohorts, and collaborating/enhancing when appropriate.

- Incorporation of environmental exposure data collection in current and future natural history cohorts. A multidisciplinary planning meeting should focus on identifying biologically plausible exposures, and adopt standards for the measurement of these exposures [i.e. PhenX (consensus measures for Phenotypes and eXposures)].

- Building informatics infrastructure and governance to support data sharing in studies of IBD.

References:


III. Report of Microbiome Workgroup

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Progress toward 2008 Global Priorities

The following global priorities and resources related to the intestinal microbiota were identified by the 2008 microbial-host interactions workgroup:

- Use genetics, immune profiles and biomarkers to predict prognosis
- Define functions of known and newly discovered IBD-related genes
- Study gene-environment interactions
- Develop a microbial “toolbox”, including a gene chip for commensal bacteria and improved bioinformatics

Significant progress has been made toward these very ambitious goals. New technologies to produce and analyze microbial data developed by the CCFA Microbiome Initiative, the National Institute of Health (NIH)’s Human Microbiome Project, and many others dramatically increased understanding of the fundamental composition and structure of the intestinal microbiota and how these enteric bacterial species and their metabolic products interact with the host to mediate mucosal homeostasis vs. chronic
intestinal inflammation. The rapid advances in high-throughput DNA sequencing and bioinformatics technology with attendant reduced costs and wider access decreased priority to develop a gene chip. Insights into genetic/immunologic/microbial interactions has flourished with identification of immunologic properties of individual species and groups of bacteria. These studies have been facilitated by gnotobiotic investigations that are evolving towards colonizations with more complex microbial communities, including those derived from healthy human subjects and patients with IBD or cocktails of human IBD-related bacterial species ("humanized" mice).

**Top Research Priorities**

1. **Define specific bacterial taxa/communities, other microorganisms (fungi, viruses), microbial gene products or metabolites associated with or predictive of the natural history of IBD, related complications and therapeutic responses.**

   Although host immune/microbial interactions are likely related to the pathogenesis of IBD, it is less clear whether the presence or absence of specific bacterial taxa or entire communities can serve as biomarkers of the natural history of these diseases, including extraintestinal manifestations and local complications, such as fistulae. High concentrations of *Faecalibacterium prausnitzii* are associated with less frequent postoperative recurrence of CD. However, the mechanisms determining this association, the specificity of this species and the clinical applications of these findings need to be explored. Although identifying fecal microbial biomarkers would be convenient, we must determine if mucosal adherent microbial biomarkers differ from those in feces.
Infectious triggers are implicated in the onset and reactivation of IBD by epidemiologic and clinical observations, but have not been widely studied. These triggers could include either enteric and systemic pathogens or functionally altered commensals such as adherent/invasive *E. coli* and mucolytic and serine protease-containing *Enterococcus faecalis*. Longitudinal studies in large, carefully phenotyped clinical populations are necessary to correlate triggers with disease flares, while investigations in inception or prediagnosis cohorts can identify factors involved in disease onset. Complementary studies in animal models could help explain phenotypic variations. Along with advances in high-throughput technologies and more sophisticated bioanalytic tools, broader profiling of microbial communities and their products by metagenomics, transcriptomics, and metabolomics will provide a more comprehensive view of microbial constituents (bacteria, archaea, fungi, and viruses) and their functions.

2. **Determine whether we can influence human disease outcomes in a durable fashion by altering the composition and function of the gut microbiota using standard therapeutic interventions, diet or fecal transplant.**

   A. **Targeted therapies.** Whether novel therapeutic interventions can permanently or consistently change human gut microbial community structure is unclear. Current strategies to alter the gut microbiome, including antibiotics, probiotics, and prebiotics, lack persistent effects after cessation of therapy. Attractive areas to explore include protective commensals adapted for the human intestinal environment, such as *Clostridium* species subsets, *F. prausnitzii*, and
Bacteroides fragilis strains, and genetically engineered probiotic strains. The duration of dietary manipulation necessary to fundamentally affect the gut microbiome is unknown. Therefore, future clinical trials, prospective cohort studies or relevant animal model experiments will need to determine whether long term dietary interventions can alter the microbiome’s composition and function. Food or food additives may be combined with microbes or microbial products to generate a stable healthy microbiome.

B. Fecal microbial transplants. Fecal microbial transplantation (fecal bacteriotherapy) from one person to another decreases recurrence of C. difficile. Preliminary studies suggest a potential benefit in IBD, particularly ulcerative colitis. Proof that fecal microbial transplantation treats IBD will provide very strong evidence that intestinal microbial contents play a fundamental role in IBD. Unanswered questions related to this approach include: What are the characteristics of the optimal recipient and donor with respect to genetics, microbiome profiles and family history of diseases such as diabetes and obesity? How should patients be prepared for transplantation? What is the optimal protocol for administering donor microbiota? Is fecal transplantation efficacious in IBD? Are the effects of fecal microbial transplantation on the composition and function of the gut microbiome permanent and if not how long do they last? What are the short and long term risks? Can susceptibility to obesity and metabolic diseases be inadvertently transferred? Are combinations (cocktail) of human commensal bacterial species designed to fill specific niches superior to feces from randomly selected donors? Answering these questions will require prospective studies of carefully phenotyped and genotyped donors and recipients following strict protocols.

3. Determine whether IBD-associated dysbiosis is a primary or secondary event.
IBD patients display a characteristic pattern of decreased complexity of enteric bacteria manifested by decreased *Clostridial* groups IV (including *F. prausnitzii*) and XIVa, and in some studies *Bacteroidetes*, with increased concentrations of *Proteobacteria* (including *E. coli*) and *Actinobacteria*. Transmission of colitis and metabolic syndrome by fecal transplants from affected to wild-type recipient mice that are normally not susceptible provides evidence for an etiologic role for abnormal commensal bacteria. However, nonspecific alterations as a consequence of the inflammatory milieu are strongly suggested by very similar dysbiosis patterns in widely diverse hosts with intestinal inflammation, including mice, humans and dogs; disparities between microbiota in active vs. quiescent IBD and similar microbiota alterations in infections, chemically and genetically-induced intestinal inflammation. This fundamental issue has profound clinical implications for proposed therapeutic strategies such as fecal transplants and dietary interventions. If dysbiosis is an important etiologic factor in IBD, programming a protective bacterial profile by intensive probiotic or fecal transplant and sustained dietary intervention could alter disease susceptibility in high risk individuals prior to onset of clinically apparent disease. However, such interventions may not be effective if dysbiosis develops as a consequence of the inflammatory process, although secondary dysbiosis could perpetuate or potentiate chronic inflammation.

4. **Better understand mechanisms by which specific intestinal microbial communities mediate chronic inflammation vs. mucosal homeostasis.**

It is critically important to determine the function of key members of the dysbiotic microbial communities of IBD patients and how relevant populations of bacteria impact mucosal homeostasis and
inflammation. Optimal animal models are necessary to interrogate evolving hypotheses. Animal models that recapitulate key genetic traits (mutations associated with IBD) are needed to better understand the impact of genetics on the mechanisms of microbiota dysfunction in IBD. This includes developing gnotobiotic simplified and complex model communities, and human fecal-associated mice with requisite IBD genetics to address key questions about microbiome function and dysfunction in IBD. Recent data suggest that such human fecal studies in mice may need to focus on colonic phenotypes as opposed to small intestine and species-specific effects of the gut microbiota on host immune function. Mouse model communities including auxotrophic mutants may be valuable tools for understanding microbial community assemblage and resilience in IBD.

5. **Identify key environmental and host genetic factors that determine the microbial composition, gene expression and metabolism of normal subjects and IBD patients and determine whether early life influences provide a critical window during which an individual’s lifelong microbiota pattern is determined.**

   A. **Nonmicrobial environmental influences.** Mechanisms by which diet, smoking history, medications, breast feeding and psychosocial stressors influences the composition and function of the human microbiome need to be determined. Also necessary for understanding gene-environment-microbiota interactions in IBD are how drugs (immunomodulatory, antibiotics, and non-IBD medications), therapeutic diets, and microbe-based interventions influence the dynamic operations of the gut microbiota in IBD. Results of these studies will guide therapeutic interventions and strategies to prevent disease onset in genetically at risk individuals.
B. Early environmental influences – a critical window? It is unknown whether an individual’s susceptibility to IBD can be altered before disease onset. Altering the gut microbiota is a possible method to accomplish this. While these studies can be more easily performed in animal models, carefully controlled epidemiologic studies are necessary to translate animal model observations to humans. Analysis of microbial effects on host epigenetic markers may also prove productive.\(^\text{13}\)

**Approaches and Resources Required to Address these Priorities**

- Serially sampling of longitudinal cohorts of carefully phenotyped, genotyped and frequently monitored IBD patients.
- Gnotobiotic facilities where IBD investigators can investigate functional influences of specific microbial species, groups of characterized cultured organisms and intact microbial communities from human and murine donors.
- Easily accessible central repositories of cultured enteric bacteria, viruses and fungi that are genomically sequenced and functionally characterized for use in *in vitro* and *in vivo* studies.
- Clinical infrastructure designed to systematically test different approaches to altering the human gut microbiota with the goal of either preventing the development of or treating IBD.

**References:**


9. Sartor RB. Genetics and environmental interactions shape the intestinal microbiome to promote IBD vs. mucosal homeostasis. *Gastroenterology* 2010 October 25;139:1816-1819.


IV. Report of Epithelial Biology Workgroup

Asma Nusrat MD (Chair), Declan F. McCole PhD (Co-Chair), Christian Jobin PhD (Co-Chair), Charles A. Parkos, MD, PhD

Progress towards 2008 Global Priorities

The following priorities and resources were identified by the 2008 IBD Epithelial Biology working group:

- Better understand factors and mechanisms that influence the intestinal epithelial barrier
- Identify the mechanisms and functional effects of microbes on the epithelial barrier
- Identify the mechanisms that control epithelial cell transformation in IBD
- Identify factors/mechanisms that promote resolution of inflammation

Significant progress has been made in the above CCFA priorities from 2008 that have advanced our understanding of mechanisms of epithelial barrier compromise, the role of epithelia in controlling the intestinal immune response and gained new insights into epithelial crosstalk with microbiota in IBD. CCFA funded projects have demonstrated changes in intercellular junction proteins (occludin, claudins, cadherins) that contribute to perturbed epithelial homeostasis and compromised barrier function observed in IBD. These studies have also increased our understanding of roles for junction proteins in epithelial homeostasis that are distinct from their primary roles in the regulation of barrier function.

It is now apparent that cytokines such as TNFα, IFNγ, IL-1β, IL-13 have potent regulatory effects on expression and function of epithelial intercellular junction proteins, polarity complexes and pattern recognition receptors that directly translate to the barrier compromise observed in IBD patients. Lamina propria lymphocytes play an important role in not only contributing to the mucosal barrier defense but also in directly modulating epithelial differentiation and barrier function.
Gene linkage studies have provided new insights into epithelial dysfunction in CD. For example, altered expression/function of junction proteins in relation to PTPN2 and/or ATG16L1 may contribute to the onset and perpetuation of chronic intestinal inflammation. Additional studies have shown an important role of TLR’s in controlling epithelial response to microbiota. RegIIIγ, a secreted antibacterial lectin maintains a bacteria-free zone that physically separates microbiota from epithelial surfaces and loss of this protection is associated with aberrant bacterial colonization and increased activation of intestinal adaptive immune responses. Furthermore, innate immune receptors such as Dectin-1 have now been linked to mucosal responses to commensal fungal microorganisms that may play a role in pathobiology of UC. It has also been shown that epithelial-specific deletion of the innate adaptor protein Myd88 in mice results in decreased expression of polymeric immunoglobulin receptor and mucin2 thereby enhancing susceptibility to certain forms of experimental colitis.

New adhesion molecules that regulate leukocyte trafficking in the gut have been described that add to our knowledge of the central role of leukocyte trafficking during mucosal inflammation in IBD. The biology of other epithelial cell types, such as Paneth cells and their roles in intestinal host defense and homeostasis has been illuminated. Importantly, dysfunction of Paneth cells is now thought to increase susceptibility to pathologic chronic intestinal inflammation as seen in IBD. Furthermore, a better understanding of the origin and function of intestinal M-cells has emerged that help in defining the contribution of these important epithelial cell types to mucosal immune responses.

There is continued progress in understanding the complex regulation of resolution of inflammation. It is now clear that resolution is not a passive process but requires lipid and protein mediators such as lipoxins and resolvins that are produced and act in a temporal fashion to resolve inflammation and
dampen its negative effects on the epithelial barrier. Manipulation of the latter agents has significant therapeutic potential for treatments directed at resolving inflammation in IBD.

The gastrointestinal epithelium serves as a selective permeable barrier that restricts access of luminal antigens to underlying tissue compartments thereby playing a pivotal role as a gatekeeper that controls overall mucosal homeostasis. The mechanisms that influence epithelial barrier function (and malfunction) are in dire need for further investigation. The outcome of such studies will have important implications in defining IBD pathophysiology and in the design of appropriate therapy for IBD patients.

**Top Research Priorities:**

1. **What regulates Intestinal Epithelial Barrier function and how does barrier compromise contribute to IBD?**

The epithelium that also includes specialized paneth cells, goblet cells and M cells is important in controlling barrier properties which is achieved by proteins in the mucous coat, intercellular junctions and secreted products. These proteins play a pivotal role in controlling epithelial homeostasis via complex signaling pathways that are poorly understood. The concerted cross talk between such pathways determines epithelial barrier function, proliferation, cell migration and cell death. Thus, questions related to identifying how epithelial homeostasis and inflammation perturb the mucosal barrier will be important in identifying contribution of these events to IBD pathogenesis.

2. **What factors prevent full blown inflammation from developing in patients/animal models with a pre-existing permeability defect?**
Increased intestinal permeability has been identified prior to the onset of inflammation in both patients and animal models of IBD, while increased permeability has also been identified in relatives of IBD patients in the absence of any clinical or histological evidence of disease. A major point of interest relates to why all individuals with a permeability defect do not go on to develop IBD. Is there an essential need for additional insults or are there regulatory factors that normally prevent manifestation of disease in individuals with a permeability defect that are dysfunctional in individuals who develop IBD? Are these regulatory factors generated by IECs themselves, immune cells or commensal bacteria?

3. How do luminal microbes cross-talk with the epithelium?

Do microbes influence epithelial homeostasis and the epithelial stem cell niche (maintenance, expansion)? What are the beneficial effects of commensal bacteria on epithelial barrier function and repair, and is this compromised in IBD? Additionally, does microbial composition compromise the epithelial barrier? Past studies have identified that bacteria can release proteases that modulate the epithelium. What is the change in microbiota composition (luminal and mucosal) and how does this contribute to the epithelial barrier compromise and wound repair in IBD? Do bacterial derived factors alter epithelial permeability in IBD?

4. How do innate sensors contribute to homeostasis and intestinal mucosa dysfunction in IBD (luminal commensal and pathogenic bacteria)?

What epithelial surface receptors and intracellular proteins sense luminal and mucosal-adherent bacteria? What is the role of pattern-recognition molecules e.g. TLR’s, NLR’s, and newly described FPRs, PGRP in the pathogenesis of IBD? While geneticists have made important contribution, very little is known about the mechanisms by which these proteins contribute to IBD. Are these epithelial-derived sensors important to contain and/or shape microbial composition? Could microbiome manipulation (pre and probiotics) influence the onset of disease? It is also unclear what the influence of diet and microbial
derived metabolites such as acetate and butyrate is on epithelial homeostasis and barrier function? Additionally, the impact of mucosal inflammation on epithelial homeostasis and survival remains to be understood.

5. How is epithelial homeostasis and wound healing regulated and altered in IBD?

Efficient repair of epithelial wounds is important in the reestablishment of the epithelial barrier and recovery of mucosal homeostasis. Such wound closure events are influenced by luminal contents and mucosal products (cytokines, protein and lipid mediators). The knowledge of factors (innate sensors/biota) and mechanisms that govern epithelial differentiation/migration and proliferation that mediate wound closure in IBD are therefore critical.

6. What epithelial derived factors regulate intestinal epithelial homeostasis and immune response?

The identification of endogenous pathways that promote the resolution of ongoing inflammation is an emerging area of intense investigation. There is continued interest in the development of proteins and lipids that serve to ameliorate inflammation. Such anti-inflammatory targets have shown promise in promoting resolution of inflammation associated with murine IBD models. Additionally, our understanding of secreted factors (cytokines, chemokines, defensins, Wnt/Wnt inhibitory proteins, mucin, trefoil peptide) in the mucosa of IBD patients that impact epithelial homeostasis (mature and stem cells) and barrier function is minimally understood and represents an important area for investigation.

7. How do epithelial uptake and paracellular- and transcellular transport pathways contribute to IBD pathogenesis and treatment?

Appropriate movement of molecules and fluids across the epithelial barrier is important for maintaining mucosal homeostasis. Can epithelial transport pathways be rescued/reactivated to reduce fluid loss
in diarrhea? Although expression of many epithelial electrolyte transport proteins is decreased in IBD, mislocalization of transport proteins can also occur. Can transport proteins involved in electrolyte fluid and absorption that are expressed be “rescued” in mild IBD to enhance fluid absorption? Are there agents that can enhance trafficking and/or retention of these absorptive proteins in the membrane and exert a functional improvement in fluid absorption? What is the contribution of IgA transepithelial transport to immune defense and IBD pathogenesis.

8. Are there environmental factors that modify epithelial barrier function (or immune status) in a manner that may modulate IBD?

Are there distinct dietary components that are associated with disease relapse in patients that compromise epithelial barrier function and thus trigger relapse? On the converse what dietary components have beneficial effects by modulating the epithelial barrier (e.g., Vitamin D). Are there specific bio-active food components that necessitate microbial-derived activities?

9. What are the relative contributions of inflammation and intrinsic epithelial growth regulatory signaling pathways to colitis associated carcinoma?

A fuller understanding of the processes implicated in the epithelial cell transformation seen in IBD is both timely and urgently needed. Do certain molecules undergo a switch in function i.e. facilitating epithelial repair but then have a role in neoplasia? Can beneficial functions be harnessed without triggering cancer related functions? What is the impact of tumor micro-environment of intestinal stem cells niche (maintenance/expansion)? How do changes in junctional proteins contribute to cancer pathogenesis?

10. Which experimental models could contribute to the generation of basic cellular and molecular understanding of IEC biology?
Murine models have significantly advanced our understanding of IBD pathogenesis. Additional novel models such as danio rerio (zebrafish) have also provided novel insights into epithelial biology, bacterial/host interaction and colitis. Could these models be exploited to decipher mechanisms of IEC interaction with bacteria, response to diverse injury and pattern of cellular interaction during disease state? Could these systems be amenable to drug screen?

10. What are the functional consequences of IBD-associated SNPs on IEC biology?

Genome wide scan association studies have identified 169 IBD candidate genes that influence susceptibility or protection to the disease. Many of these genes are expressed in the intestinal epithelium. To what extent do these polymorphisms impact normal IEC function? Do these SNPs functionally modify gene expression/function? Given our understanding that many genes are affected in IBD and that even the most prominent individual candidate genes have a limited distribution across the spectrum of IBD patients, another key area of understanding will be the study of how SNPs in multiple candidate genes contribute to dysfunction of key regulatory events in IECs i.e. barrier function, bacterial sensing, autophagy etc.

In summary, the following are the measurable outcomes that will arise from these research priorities:

- Definition of signaling pathways linked to known and newly discovered barrier forming proteins that regulate epithelial homeostasis (barrier, migration, proliferation, differentiation)
- Identification of positive and negative influences of leukocytes/microbes on epithelial homeostasis and the precise subsets of leukocytes/microbes responsible
- Definition of specific environmental or pharmacologic factors and their interplay that disrupt epithelial homeostasis and contributions to disease in normal and genetically susceptible patients and experimental models
Definition of which molecules undergo a switch in function (i.e. facilitating epithelial repair but then may also have a role in colitis associated neoplasia).

- Specific contributions of stem cell-associated and junctional molecules
- Identification of studies that validate new models (drosophila melanogaster, zebrafish, C. elegans)
- Establishment of microbial composition (and related metabolism) that regulates epithelial homeostasis

**Approaches and Resources Required to Address these Priorities**

- Animal models
- Techniques for culture of native intestinal epithelium and organoids
- Cell line bank
- Screening strategy to identify epithelial relevant “hits” using lower vertebrate model (e.g. zebrafish)

**References**


V. Report of the Innate Immunity Workgroup

Thaddeus S. Stappenbeck, MD, PhD (Chair), Clara Abraham, MD (Co-Chair), and Scott E. Plevy, MD (Co-Chair)

Progress towards 2008 Global Priorities

The following global priorities and resources were identified by the 2008 Innate Immunity working group:

- Identify the relative contribution of cells of the innate immune system to maintenance of intestinal immune homeostasis
- Identify the specific signaling pathways of the innate immune system that regulate mucosal health
- Identify the mechanisms by which signaling events evoked by innate immune receptors are integrated within the cell and lead to alterations in protein and gene expression
- Identify all peptide and nonpeptide mediators/regulators of inflammation secreted by innate immune cells
- Determine the unique and redundant mechanisms by which the innate immune system responds to commensals and pathogenic microorganisms
- Characterize the mechanisms by which the innate immune system regulates the adaptive immune response and vice versa

Over the past 5 years, research in the field of innate immunity and mucosal immunology has been an area of rapid movement, with potential for profound impact on IBD research in the next 5 years. In addition to further uncovering novel functions/regulation for previously recognized innate immune cells, the major surprise is that we are still discovering and characterizing novel innate immune cell types such
as innate lymphoid cells (ILCs)\textsuperscript{1}. Recent advances in other fields such as genetics and microbiological analysis have also immensely affected the direction of innate immunity research in IBD. Functions of some of the genes in the over 100 loci that have been associated with IBD identified primarily through genome-wide association studies (GWAS), have begun to be uncovered within specific innate immune cell types such as monocyte-derived cells and Paneth cells. Our understanding of microbial-associated molecular patterns (MAMPs or PAMPs) has significantly expanded our knowledge of their related signaling pathways. In addition, the massive expansion of knowledge of the intestinal microbiome has helped us identify potential microbes that normally interact with the innate immune system.

**Top Research Priorities**

1. Define new roles of known innate immune cell types (Paneth cells, goblet cells, neutrophils, macrophages, dendritic cells, natural killer (NK) cells) and define the roles of emerging innate immune cells (lymphoid tissue inducer (LTI) or mucosal associated invariate T (MAIT) cells).

The traditional innate immune cells include monocyte-derived cells such as macrophages, dendritic cells, neutrophils and epithelial cell types, such as defensin-producing Paneth cells and mucus-producing goblet cells. Additional insight into unique subpopulations and functions within these traditional innate immune cells continues to be an area of active and important investigation. A major area of expansion has been driven by key findings from numerous labs that have identified novel ILCs that play important roles in the innate immunity of in the intestine \textsuperscript{1}. The ‘old’ ILC is NK cells (now also known as ILC1). Recently discovered ILCs include lymphoid tissue inducer cells (may be two populations including IL22 producing NK22 and IL17 producing ILC17) and ILC2 (a.k.a. nuocytes) that produce IL-4 and IL-13. Most
surprisingly and excitingly, this repertoire of innate lymphoid lineages appears to mirror the attributes of related T helper lineages.

2. How do IBD susceptibility polymorphisms and the emerging IBD gene mutations affect innate immune function and intestinal immune homeostasis? Do these mutations identify specific pathways that can be explored experimentally and therapeutically?

Many of the implicated IBD loci are in regions that include genes important in modulating innate immune responses. Significant recent progress has been made in understanding the role of a number of the implicated innate pathways in intestinal immune homeostasis, and the functional consequences of certain common polymorphisms, such as those in NOD2, ATG16L1, IRGM, PTPN22, IRF5 and IL-23R\(^2\). A major challenge is that many loci are associated with genes of unknown or unclear function and must be evaluated in terms of innate immune function. We must therefore: 1) identify the specific gene(s) in the implicated regions that is contributing to the association; 2) understand the contribution(s) of the gene to intestinal immune homeostasis under physiological conditions; 3) define the specific consequences of the implicated polymorphisms and the mechanisms wherein these polymorphisms confer either protection from or risk for IBD; and 4) identify how these genetic associations can assist in the diagnosis, prognosis and therapy of IBD.

3. What is the nature of the crosstalk of host innate immune cells with the microbiome?

Intestinal innate immune cells consist of various unique subsets, with many demonstrating distinct features relative to immune cells that circulate or reside in other tissues. For example, intestinal

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macrophages secrete very low levels of pro-inflammatory cytokines upon stimulation with microbial ligands through pattern recognition receptors (PRR), yet are more efficient at phagocytosis and microbial killing than peripheral macrophages \(^3\). We have hints as to why this occurs, but a definitive understanding of this process is still not complete. This regulation is likely lost in IBD, so understanding the tolerance to intestinal microbes is a fundamental issue. Our lack of understanding in this area is, in part, related to the fact that we do not yet know all the recognition systems for MAMPs. For example the number of LRR-containing molecules is much greater that the molecules we have identified to date with microbial-recognition capacity. This is an example of just one of the microbial recognition motifs. Therefore, additional studies in mouse models and human cells will be needed to fully define the various responding receptors—and understand their behavior in the periphery versus the intestine.

That resident intestinal microbiota are tolerated, but pathogenic microbes are targeted by host defense mechanisms is increasingly recognized as over-simplifying the dynamic, ongoing host-microbe dialogue within the intestine. The immune system continuously monitors resident intestinal microbiota, and select antimicrobial mechanisms are constitutively engaged to prevent overgrowth and maintain homeostasis of colonizing microbes. It is not yet understood how the intestinal immune system determines when to respond in an inflammatory fashion to either resident microbiota or pathogens \(^4\). This again is a fundamental question and inroads into understanding this concept will have a profound impact on IBD.

**Approaches and Resources Required to Address these Priorities**

- To quickly advance research in this area, we need to develop specific markers with which to identify the various subpopulations of both mouse and human innate cells. We need to develop
tools/approaches that allow for increased ease of isolating these populations from intestinal tissues in vivo.

- For mouse studies, there is a pressing need for better and more specific tools to dissect the role in vivo of these different cell types. This includes Cre systems to test functional molecules by lineage knockouts (KO) and lineage tracing tools to test the development and inter-relationship of these lineages in vivo. This is particularly critical as many of these lineages are defined by activity that is dependent on the microbiota.

- For epithelial lineages there is a need to perfect systems to grow primary and ES-derived intestinal epithelial cells. Such tools will facilitate our understanding of the specific function of these molecules related to the genetic and environmental influences that have been recognized for IBD.

- We need to identify the degree to which the well-characterized mouse innate subpopulations accurately reflect human innate cell subpopulations. This will be critical to direct specific efforts towards IBD.

- We need to develop mouse models allowing for additional mechanistic studies. Not only are gene KOs and lineage KOs important to infer function both globally and in specific cell types, but also lineage tracing systems to evaluate the cell-specific expression in health and disease states as well as knockins of specific polymorphisms.

- Expanded studies of genotyped human tissues and cells must take place. The limited access to tissues and cells restricts the functional readouts that can be conducted; high throughput approaches for small sample sizes and for multiple different functional readouts will be essential.

- We need to develop methods for uniform sample processing and standard operating techniques to minimize the variation introduced and enhance the variation attributable to the genetic polymorphisms.
We need to establish large cohorts of well-genotyped individuals that can be recalled for subsequent functional studies, along with pooling of these resources at multiple locations to allow for ascertainment on uncommon alleles.

It will be important to understand the pathway's role in the balance between our ability to combat infection and intestinal immune homeostasis.

Additional mice in which KO of a repertoire of responding PAMP receptors are needed. This will allow for advances in more fully defining the various responding receptors—and understanding their behavior in the periphery versus the intestine.

As we begin to understand which microbes are present in health and disease, the real question becomes what changes matter functionally. This is an important challenge for the field moving forward. As we understand the consequences of perturbations in specific innate immune pathways on microbial ecology (including viral infection), we need functional models to test these ideas. The translation of these models to either germ free settings or careful attention to the microbiota (including endemic viruses) is a second substantial hurdle. Surprisingly, it is sometimes the low abundance species that have been shown to effect pathology in animal models in the oral cavity and the intestine. In addition, some relatively abundant species don't increase in abundance after triggering IBD in genetically susceptible models.

An important goal will be to define mechanisms regulating human intestinal innate responses, translate the high impact microbial communities into human intestinal innate cell outcomes, and ultimately, define the dysregulation in host-microbial interactions in IBD.

References:


VI. Report of Adaptive Immunity Subcommittee

Theresa T. Pizarro, PhD (Chair), Edwin F. de Zoeten, MD, PhD (Co-Chair), Scott Snapper MD, PhD, Matthew B. Grisham, PhD, and Dmitry V. Ostanin, PhD

Progress towards 2008 Global Priorities

The following areas of investigation were previously identified as global priorities to move the field of adaptive immunity in IBD research forward:

- Phenotypically and functionally identify intestinal T-effector cells and evaluate their precise role, particularly of the Th17 subset, in IBD
- Determine how the interaction between the gut microbiota and innate immune cells alters adaptive T-cell responses in IBD, and identify specific microbial antigens that stimulate/activate T-effector and T-regulatory cell (Treg) function in the gut
- Understand the mechanism(s) of Treg induction, particularly as it applies to potential treatment modalities for patients with IBD
- Development and application of novel mouse models, including T-effector and Treg reporter mice, for which novel methodologies (e.g., Cre-lox approaches) may be required

Significant progress has been made in several of these identified areas, while a greater initiative is needed in others within the next five years. For example, the role of Th17 effector cells in the pathogenesis of experimental IBD appears to be much more complicated than initially observed, with
some studies suggesting a pathogenic role, while others demonstrating a protective or regulatory role, depending upon the specific mouse model employed (1). In support of this concept, results from a recent clinical study reported that selective blockade of IL-17A (secukinumab) was ineffective in the treatment of CD, and may even exacerbate disease in a subset of patients (1). In regards to the relationship between the microflora and gut immunity, the interaction of the intestinal microbiota/innate immune cells with the mucosal adaptive immune system has been reported to play an important and required role for the development of Th17 cells. In fact, germ-free raised mice were shown to be deficient in IL-17-secreting CD4⁺ T-cells (2), which has also been described in toll-like receptor (TLR)9 deficient mice (3). Furthermore, Th17 development appears to be augmented by the TLR5 ligand, flagellin (4). Other groups have taken on the task of defining specific microbes, or phyla of microbes, that are important for activating Th17 immune responses. To this end, segmented filamentous bacteria (SFB) were noted to be important for the development of T helper cells (5). This group of bacteria was noted to be important for the reconstitution of Th17 cells in mice that lacked this immune cell subset (6) and were also shown to be important for protection against *C. rodentium* infection, suggesting that stimulation of Th17 is required for the beneficial, as well as the pathogenic, effects of gut immune responses. Interestingly, Tregs within the periphery have been reported to differentiate into IL-17-producing Tregs (7). However, it is unclear if this phenomenon (i.e., development and/or expansion of IL-17⁺ Tregs) occurs within gut-associated lymphoid tissues (GALT) or is dependent on TLR signaling and/or exposure to the gut microflora, which may ultimately lead to uncontrolled, chronic intestinal inflammation. In human studies, a recent report has identified lamina propria IL-17⁺Foxp3⁺ CD4⁺ T-cells that are preferentially expressed in the inflamed gut mucosa of CD patients, share phenotypic characteristics of Th17 and Treg cells, and display potent *in vitro* suppressor activity (8). Clearly, although significant progress has been made within the last five years in identifying potential mechanisms involving the adaptive immune system in the pathogenesis of IBD, much more
work remains to be done, particularly in translating this knowledge into clinically relevant applications and developing appropriate tools (i.e., novel animal models) to address emerging questions in this field of investigation.

**Top Research Priorities**

A consensus was reached that further efforts are needed to define the precise phenotypic and functional properties of T-effector and Treg/emerging regulatory subsets that distinguish different forms of IBD and clarify the current paradigm of Th1/Th2/Th17 for CD vs. UC. In addition, emphasis was placed on translating concepts generated from experimental models of IBD to the clinical setting by identifying potential biomarkers for active disease, characterizing novel cytokines/mediators that are involved in IBD pathogenesis and may be targeted for potential therapeutic modalities. In addition, an urgent need was identified for developing humanized mouse models. The top overarching research priorities are summarized below:

**1. Further define the role and function of Tregs and other regulatory cell populations in the pathogenesis of IBD.**

Foxp3⁺CD4⁺CD25⁺ Tregs that maintain immunologic homeostasis were initially considered to be a homogeneous population of naturally occurring, thymus-derived CD4⁺CD25⁺ cells or natural Tregs (nTregs). However, other classes of Tregs are induced (inducible Tregs or iTregs) in the periphery from effector lineage CD4⁺CD25⁺Foxp3⁺CD127high T-cells, either by IL-10 or TGFβ, or by association with activated CD4⁺CD25⁺Foxp3⁺ Tregs. These subsets differ in their antigen specificities, in T-cell receptor
signal strength, and in co-stimulatory requirements needed for their generation, suggesting that nTregs and iTregs may have different roles in adaptive immune responses. However, whether iTregs have any unique function(s) in IBD compared to nTregs is not yet clear. Therefore, clarifying the specific phenotypic and functional properties of nTregs vs. iTregs, as well as other emerging regulatory cell populations, including myeloid-derived suppressor cells (MDSCs), Bregs, and mesenchymal stem cells (MSCs) and their role in IBD is an important area of investigation.

The commensal microflora, through TLR engagement, can limit Treg conversion and induce production of IL-17 from T effector cells, i.e., Th17 cells (3, 9). Indeed, TLR activation by commensal bacterial products has been shown to induce intestinal Th17 cells (3, 6). Interestingly, Tregs within the periphery have also been reported to differentiate into IL-17-producing Tregs (7). However, it is unclear if this phenomenon (i.e., development and/or expansion of IL-17+ Tregs) occurs within GALT or is dependent on TLR signaling and/or exposure to the gut microflora, which may ultimately lead to uncontrolled, chronic intestinal inflammation. As such, it will be important to understand the specific functional role of mucosal IL-17-producing Tregs in the pathogenesis of IBD.

An emerging area of investigation is the impact of epigenetics on Treg function. In fact, epigenetic modifications suggest a mechanism for maintenance of stable expression of the foxp3 gene, yet other data suggest there may be multiple mechanisms controlling plasticity (10,11). In regard to clinical applications, several laboratories have demonstrated that pharmacologic targeting of these epigenetic mechanisms can increase Treg number, function and stability both in vivo and in vitro. However, a better understanding regarding the role of methylation and acetylation is needed to insure that appropriate targets are defined in IBD.
2. Characterize the identity and specific function(s) of pathogenic mucosal T-effector cells and clarify the Th1/Th2/Th17 paradigm and its application to IBD.

A significant proportion of CD4+ effector T-cells that reside within the inflamed bowel appear to be longed-lived memory T-cells that continuously recirculate through the blood, non-lymphoid tissue (including the gut), and lymphatics. Historically, it has been thought that disease-producing effector cells are short-lived CD4+ T-cells that are continuously generated from naïve CD4+ cells via their interaction with enteric antigen-loaded dendritic cells (DCs). New and provocative data suggest that a significant proportion of interstitial T-cells in the inflamed gut are in fact self-replicating, memory T-cells that have the ability to exit the tissue via the draining afferent lymphatics and enter the systemic circulation where they continuously recirculate to a variety of different tissues, including the bone marrow, liver, lung and gut (12,13). In fact, it has been demonstrated that these CD4+ effector cells possess many of the same properties as hematopoietic stem cells (14). Obviously, the presence of long-lived, self-renewing, antigen-specific memory T-cells within the systemic circulation, as well as peripheral tissues (including the gut), would have remarkable therapeutic implications for the treatment of intestinal as well as extra-intestinal inflammation in patients with IBD (15). As such, further research initiatives into this area of investigation are warranted.

While the Th1/Th2 paradigm has been used for over two decades to describe CD4 T-cell effector function in many organ systems, including the GI tract, the more recent discovery of the Th17 lineage has revolutionized our global understanding of immune regulation in health and disease, specifically in the pathogenesis of IBD. However, although CD has been generally attributed to Th1 and Th17 effector
functions, and UC more loosely linked to Th2 effector function, emerging evidence suggests that these associations are not so clearly delineated and are highly dependent on the temporal (i.e., early vs. late) and phasic (chronicity) state of disease in the pathogenesis of both CD and UC. Interestingly Th17 development is dependent on the pleiotropic cytokine, TGFβ, which is also required for the development of Tregs, establishing an important link between Th17 and Treg development and function. In regard to IBD, it would be advantageous to establish whether the Th1/Th2/Th17 paradigm or another classification system can be utilized to distinguish patients or subsets of patients with CD vs. UC.

3. Investigate (novel) cytokines affecting mucosal adaptive immunity and their potential translational applications.

One of the fastest moving areas in inflammation and immunology has been the identification and characterization of the ever-expanding list of cytokines and the role they may specifically play in maintaining normal gut homeostasis and in chronic intestinal inflammation. The area of cytokine biology has also been one of the major targets of interfering with disease processes, with direct translational applications, and has had great prior success in the clinical arena. Interestingly, the most recent findings in this area of investigation are that novel family members of classic proinflammatory cytokines, such as IL-1 and TNF, are emerging as critical factors in IBD development. These include the novel IL-1 family members, IL-33, which has been implicated primarily in the pathogenesis of UC (16-19), and IL-37, a potent anti-inflammatory cytokine with the ability to downregulate DSS-induced colitis (20). In addition, other IL-1 family members, including IL-36γ, IL-36δ, and IL-36ε and their antagonist, IL-36Ra, as well as IL-38 have all been shown to markedly modulate either Th1/Th2/Th17 (of combinations of)
immune responses (21, 22). Similarly, the TNF family member, TL1A (TNFSF15) and its receptor, DR3, have been shown to co-stimulate T-cells to promote expansion of both effector T-cells and Tregs, as well as induce both Th1 and Th17 immune responses (23, 24). Furthermore, recent studies have reported that TL1A has the ability to drive IL-13-dependent small intestinal inflammation, implicating its role in Th2 dependent pathways (25), as well as fibrostenosing disease (26, 24). TWEAK, another member of the TNF superfamily, and its receptor, Fn14, have also been recently reported to modulate IL-13 with the ability to infer damage to intestinal epithelial cells and cause mucosal inflammation (27, 28). Furthermore, increased levels of TWEAK, Fn14, as well as IL-13 in the gut mucosa of UC patients is associated with the severity of disease (28). Further investigation is needed into the specific, mechanistic contributions of these novel cytokines to IBD pathogenesis and if they serve as potential targets for therapeutic interventions.

Approaches and Resources Required to Address these Priorities

- Support is needed for dentification and utilization of novel mouse/animal models that may better represent human IBD
- Support is needed for development of ideal humanized mice.
- Investment in a humanized mouse repository is needed, perhaps with a particular microbiome.

References


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VII. Report of IBD Diagnoses Subcommittee: Clinical Classification and Prognostic Models

Lee A. Denson MD (Chair), Corey A. Siegel MD, MS, (Co-Chair), Peter D. R. Higgins MD, PhD (Co-Chair)

Progress towards 2008 Global Priorities

The following global priorities and resources were identified by the 2008 IBD Diagnoses working group:

- Use genetics, immune profiles, and biomarkers (clinical, medical, genetic) to predict individual prognosis (natural history, response to therapy, toxicity of therapy).
- Better understand age- and sex-specific risks versus benefits of established and new medical and surgical therapies
- Conduct a workshop to focus on defining prognosis/phenotypes
- Establish a pediatric research network

Significant progress was made with regard to these global priorities and resources since 2008. A Pediatric IBD Research Network named the Pediatric Resource Organization for Kids with Intestinal Inflammatory Disorders (PRO-KIIDS) was formed with CCFA support. This network now includes 42 Children’s Hospitals in the United States and Canada. The first project for the network, RISK, supported by a CCFA research initiative, will use clinical criteria, genetics, immune profiles, and intestinal gene expression patterns to predict individual prognosis (natural history, response to therapy, toxicity of therapy). Enrollment of the cohort was completed in 2011, and prospective three year follow-up is ongoing. A series of three meetings took place in 2009 in order to define a new Paris classification system for pediatric-onset IBD\(^1\). This system can be used in conjunction with the existing Montreal classification system. The two main additions include definition of very early onset IBD as < age 10 at
onset, and early onset IBD as < age 17 at onset. In addition, modifiers for extensive small bowel CD, growth failure, and severe UC were defined. A consensus definition for “early” CD was also derived and validated over the past two years\(^2\). The primary features include duration of disease of not more than 2 years, no significant GI dysfunction or fibrotic or penetrating complications, and no exposure to immune modulators or biologics. This definition could be used to define a more homogenous patient population for clinical trials of new disease-modifying therapies. While several reports since 2008 have sought to define risks for rare adverse events of medical therapy including lymphoma, these data remain limited with regard to patient-specific risk factors\(^3\). Ongoing cohort and registry studies will seek to better define these long-term risks.

**Top Research Priorities**

It was felt that the most important research questions in the area of IBD Diagnostics currently have to do with developing better ways to classify patients in terms of predicting natural history, response to therapy, and adverse effects of therapy. A summary of the top priorities identified follows.

1. **Conduct large adequately powered prospective cohort studies to validate clinical, genetic, immune, microbial, or tissue gene expression factors that predict those at risk for rapidly progressive or severe disease or IBD-associated complications.**

The association between \(NOD2\) genotype, antimicrobial serology (AMS), and higher risk for stricturing/penetrating behavior in CD has been reported in several adult and pediatric studies, and a clinical prognostic panel based upon \(NOD2\) and AMS is now commercially available\(^4,5\). However, the positive predictive value (PPV) of a prognostic tool limited to this factors remains relatively low, in particular for prediction of complicated disease behavior in CD over the next three years\(^6\). It will be
important to test additional clinical, genetic, microbial, and serologic markers including cytokine auto-
antibodies for their ability to improve the PPV of the current panel\textsuperscript{6}. Several recent studies have shown
that the use of intestinal or peripheral blood mononuclear cell (PMBC) gene expression panels may
provide an accurate means to predict response to therapy\textsuperscript{7-9}. It will be critical to test these as predictors
of disease course and treatment response, in adequately powered and validated studies, in addition to
the current biomarkers. Proteomic analysis of existing plasma banks may also identify more powerful
biomarkers and should be pursued. The RISK prospective cohort study will provide the platform to test
and validate these biomarkers for pediatric CD. A similar prospective cohort study would be required to
do the same for pediatric UC. This study, termed PROTECT (Predicting Response to Standardized Colitis
Therapy), has been funded by NIH utilizing the PRO-KIIDs infra-structure and will begin enrollment in
July of 2012. While it is likely that results in pediatric-onset disease will apply to adult-onset disease, it
will be critical to test models developed in RISK and other pediatric-onset cohorts in adult-onset
patients.

\textbf{2. Test accuracy of current diagnostic imaging modalities which minimize exposure to ionizing
radiation in monitoring inflammation and/or fibrosis, and determine whether these can predict
clinical outcomes and responsiveness to therapy. It will be critically important to test for cost
effectiveness of these modalities compared to serum, stool, or endoscopic monitoring methods.}

There is a growing trend in which imaging is changing from solely a diagnostic method to a disease
monitoring tool. While barium radiography was the mainstay of IBD imaging for many years, it has been
displaced in modern practice by CT enterography (CTE) and MR enterography (MRE). Research studies
over the next few years should focus upon addressing the above questions using enterography\textsuperscript{10}. Cross
sectional imaging is the state of the art technique, which can detect and localize inflammation, in
addition to identifying bowel obstruction and perforating complications. These imaging modalities, given
their cost, must be proven to be superior to existing low cost methods of monitoring disease activity in patients. An adjunctive method for imaging perianal fistulas in centers with local expertise is rectal EUS. There are several contending imaging techniques in development/early application to IBD, which may complement existing modalities. These include: PET-CT, MRI with various new sequences/delayed washout, Magnetization Transfer MRI, Contrast enhanced ultrasound, and Ultrasound elastography/Shear wave velocity imaging.

Ideally, study designs will be prospective, and will compare modalities in their ability to predict clinical outcomes and responses to therapy. Study designs in imaging should conform to the STARD guidelines for design of diagnostic studies (http://www.stard-statement.org/).

3. Develop comparative effectiveness (CE) studies to test competing strategies for disease monitoring, treatment and cost-effectiveness.

The implication of biomarker and diagnostic imaging based disease monitoring is that patients judged to be at higher risk for rapid progression of bowel damage may benefit from earlier introduction of biologic therapies more likely to induce mucosal healing. In terms of cost effectiveness and both clinical practice and clinical trial endpoints, it will be important to determine whether a fecal biomarker such as calprotectin may serve as a surrogate endpoint for mucosal healing defined by either diagnostic imaging or colonoscopy. Large cohort studies will be needed to define results for diagnostic imaging or fecal biomarkers which predict future clinical relapse and surgery, as well as to model the effect of early introduction of corticosteroids, immune modulators, and biologics on these outcomes and test these using comparative effectiveness methodologies. Ultimately, a clinical trial of early biologic therapy would be required to conclusively show whether this will modify disease course in high risk patients. In this regard, analyses of patient genetics and biomarker profiles from clinical trials of existing and new therapeutics would be extremely valuable.
4. Develop tools to aid in shared medical decision making between providers, patients and parents.

When other research identifies effective models for disease behavior, monitoring, and treatment strategies, we still need to be able to communicate this to our patients and parents so that they will accept recommendations for care.\textsuperscript{16} Tools have already been developed, but need expansion to broadly cover the multiple decisions made by IBD patients and their providers.

**Approaches and Resources Required to Address these Priorities**

- Support is needed to complete the planned three year follow-up on all subjects in the current RISK cohort, and thereby to derive and validate the model for complicated behavior and early surgery. Ideally, additional support will then be obtained to continue follow-up of this cohort, to measure additional important long-term clinical outcomes and adverse effects of therapies.

- Support for a similar longitudinal cohort comprised of adult-onset IBD patients will need to be established to validate findings from the pediatric RISK and PROTECT cohort studies. A common infra-structure for data management and biobanking of these large cohorts would be of great benefit in reducing overall cost and facilitating new studies.

- To achieve the priorities related to Diagnostic Imaging, multicenter prospective comparative studies of strategies for monitoring intestinal inflammation and fibrosis in IBD will be needed. Industry-sponsored studies for MR enterography in adult and pediatric CD have recently started. It will be critical to come to consensus on one MR enterography scoring system for use in clinical practice in both adult and pediatric CD patients, and potentially as an end point in clinical trials. It will be important that these studies formally test the utility of less costly approaches, such as fecal biomarkers of inflammation.
● The evaluation and treatment modalities are available to perform CE studies, but for such studies significant resources would be needed for study design and implementation. The Clinical Research Alliance and PRO-KIIDs could be a perfect setting for these types of studies – but would likely need to take a similar approach to MERIT UC and PROTECT by seeking NIH or other federal funding.

● Support is needed to develop effective decision aids, and to test these decision aids in practice to make certain that they are easy for patients/parents to understand, and to determine if they indeed help in medical decision making and ultimately in patient outcomes.

Impact upon Patient Outcomes

If accomplished, these priorities will have a significant impact upon improving patient outcomes. A diagnostic tool which will accurately predict which patients are most likely to experience an aggressive disease course would allow for better targeting of disease-modifying biologic therapies to those who are likely to derive the greatest benefit. These therapies might then be more likely to prevent bowel damage and the need for surgery, if offered earlier in the disease course as guided by this diagnostic tool. Conversely, patients predicted to have a more benign course could defer the use of these therapies unless dictated by current symptoms and failure of first-line approaches. In the current economic environment, it will be critically important to have a better understanding of both the clinical effectiveness, and cost, of different treatment options in individual patients. The proposed comparative effectiveness studies will fill this knowledge gap. Finally, it will not be clinically useful to have this new information regarding disease prognosis and comparative effectiveness of different treatment approaches unless it can be communicated to patients in an effective way. This need will be met by the development of patient-based decision aids.

References


Progress towards 2008 Global Priorities

The 2008 IBD pediatric and adult working group identified the following top global priorities and resources:

- Identify clinical or biological variables that predict treatment outcome and risk stratification according to these variables in pediatric IBD
- Identify the incidence of short- and long-term adverse effects of medical therapy used to treat pediatric IBD
- Better define the risks of medical and surgical therapies in IBD
- Better understand age- and sex-specific risks and benefits of established and new medical and surgical therapies

Progress has been made towards each of these goals. From November 2008 through June 2012 over 1200 patients < 17 years of age with newly diagnosed CD were enrolled in the CCFA sponsored RISK Stratification Study which is examining the relationship between genetic, serologic, and microbiologic factors and clinical course over 3 years. One-year follow up is already available on over half of this inception cohort. In 2012, The PROTECT Study (“Predicting Response to Standardized Pediatric Colitis Therapy”) was funded by NIH and, similar to RISK, will examine the relationship between genetic, serologic, microbiologic and other factors and the likelihood of children newly diagnosed with ulcerative colitis entering and being able to maintain remission on mesalamine only, and avoiding the need for additional medications with an increased risk of serious side-effects.

There are both independent national multicenter registries as well as the pharmaceutical based DEVELOP registry sponsored by Janssen Ortho-Biotech that are examining patient safety. In the national
registries that are directed by groups of academic pediatric IBD centers, there are currently about
10,000 pediatric patients followed. In the DEVELOP registry started in late 2008, there are currently
about 4000 children with IBD enrolled, approximately half of whom have received anti-TNFα therapy.¹
Meaningful data will only come with further years of observation.

Controlled clinical trials, including SONIC (Study of Biologic and Immunomodulator Naïve Patients in
Crohn’s disease) and COMMIT (Combination of Maintenance Methotrexate-Infliximab Trial) did not
reveal a generally significant increased risk for neoplasia and infections in IBD patients treated with anti-
TNF therapy or a combined anti-TNF therapy and an immunosuppressive agent (azathioprine or
methotrexate) for up to 12 months². Additionally, an analysis of several study cohorts with
autoimmune diseases found no association between anti-TNF therapy and a higher risk for serious
infections³. However, there is no doubt that a small percentage of patients experience serious adverse
reactions or infections due to anti-TNF therapy such as tuberculosis, pneumonia, psoriasis and
demyelinating diseases. Further studies are necessary to be able to better identify these groups of
patients.

Retrospective single center analyses have revealed no increased risks of postoperative infectious
complications in CD patients treated with anti-TNF therapy⁴. The studies in patients with UC and those
exposed to preoperative anti-TNF therapy are equivocal. Prospectively collected larger cohort studies
are needed.

The CCFA funded PIANO (Pregnancy in Inflammatory Bowel Disease and Neonatal Outcomes) study has
successfully included more than 1000 newborns. Preliminary results reveal that there are no significant
infectious risks or risks for malformations in newborns of mothers treated with anti-TNF and/or
azathioprine/6-MP therapy⁵.
Currently a prospective multi-center study is ongoing by the Clinical Research Alliance of the CCFA to study the risks of perioperative infections and immunosuppressive therapy. Additional efforts are necessary to better define risk/benefit ratios especially in young (<20 years) and older (>60 years) patients with IBD treated with biologics or combination therapy.

**Top Research Priorities**

1. **Individualization of anti-TNF therapy in IBD**

A significant number of patients are primary or secondary non-responders to anti-TNF therapy. One of the reasons seems to be the clearance of anti-TNF antibodies and the development of antibodies to the anti-TNF agents. It would be ideal if patient customized dosing frequencies and adjustments for anti-TNF drugs could be applied to improve therapeutic success and prevent loss of response.

2. **Prediction models for the disease course and monitoring of disease activity**

Thus far, we do not have reliable markers to individually predict and monitor the course of disease in patients with CD and UC. Taking guidance from the project initiated by the International Program to develop New Indexes in CD (IPNIC) group, studies should focus on defining composite scores (analogous to the model for end-stage liver disease (MELD) score in hepatology). Studies or substudies associated with large clinical trials, similar to the more recently reported impact of mucosal healing on the clinical course of CD and UC, are needed to identify clinical, endoscopic, serologic, fecal and genetic markers which may further predict disease course or disease complications. These same factors
may also show utility in monitoring the therapeutic efficacy of individual drug regimens. These markers should then be validated in large prospective cohorts.

3. Develop instruments to objectively validate CD activity in clinical trials

There are substantial shortcomings of the current “gold-standard” for the evaluation of the clinical efficacy of potential new CD drugs, the Crohn’s disease activity index (CDAI). Principally, the subjectivity of the reported clinical symptoms in the index is problematic. These symptoms are not specific for CD, and can also be observed in patients with irritable bowel syndrome with no underlying overt intestinal inflammation. For a more reliable evaluation of new drugs or comparative effectiveness studies in CD, new indices with objective markers of disease activity need to be established.

4. Determine when therapy can be “stepped-down” from combination therapy with biologics +/- immunosuppressants

Combination therapies seem to be more effective in most CD patients compared to a mono-agent approach. However, combination therapies have a higher potential for adverse events and development of neoplasia. There is only limited information available about the continuing disease course in IBD patients after a switch to a single agent maintenance therapy after being in stable remission on a dual therapy with e.g. azathioprine and an anti-TNF agent for longer time periods. Markers need to be established for patients in remission on dual therapy to predict high likelihoods for sustained low disease activity on a de-escalated single drug maintenance drug regimen.
Specific pediatric challenges

1. What is the ideal way to use biologic therapy in the treatment of pediatric Crohn’s disease?

Children often present with more extensive and severe disease. Moreover, growth impairment is an important factor in determining initial and follow-up therapies. Although regimens using immunomodulators have been successful in controlling disease, immunomodulators at best work in 50% of cases and do not seem to improve growth adequately. Prospective data are needed to better understand outcomes with the use of anti-TNF drugs as primary therapy, either alone or in combination with immunomodulators. Which immunomodulators are safest when used in conjunction with anti-TNF agents? Are there certain high risk populations for which combination therapy is a relative contraindication?

2. What is the ideal way to monitor success of our therapy?

The pediatric IBD population is in need of prediction models for disease course and monitoring of disease activity; similar to the need in the adult IBD population. This leads to further questions including: what is the benefit vs. risk of striving for mucosal healing versus symptomatic remission in children with IBD? Should the long horizon of disease duration in pediatric patients influence the zeal with which mucosal healing should be pursued?

3. Can we develop new treatment strategies for the treatment of fulminant ulcerative colitis?

Colectomy rates in children with severe colitis requiring intravenous steroids and subsequent infliximab therapy are still very high. A prospective study with individualized infliximab therapy guided by serum infliximab levels should evaluate a tailored approach for this patient group in which infliximab
pharmacokinetics appears to be different than in adults. Is there still a role for calcineurin inhibitors in this population?

Approaches and Resources Required to Address these Priorities

- Most of the above challenges can only be solved in the context of large prospective clinical trials or registries. The CCFA Clinical Research Alliance and the CCFA Pediatric Resource Organization for Kids with Inflammatory Intestinal Disorders (PRO-KIIDS) network can provide parts of the infrastructure for such endeavors, but funds are limited. Therefore additional funds (e.g. from NIH sources) might be necessary.
- There are several potential synergisms, which should be considered in the future planning of trials/registries. Protocols should be developed, which can be applied in both pediatric and adult patients or by collaborating with other large clinical research networks such as the GETAID or the Swiss IBD cohort.
References


